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Short communication

Δ^9-Tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study

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Abstract

Cannabinoid modulation of prefrontal cortex and hippocampus neuronal functioning has been correlated to the disruptive action of marijuana on memory tasks. This study investigates the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on dopamine, glutamate and GABA levels in vivo by brain microdialysis in the prefrontal cortex. Δ^9-THC (1 mg/kg, i.v.) significantl increased extracellular dopamine and glutamate levels and decreased GABA levels. These effects were prevented by the cannabinoid antagonist SR141716A (1 mg/kg, i.v.), which per se was ineffective. These results suggest that Δ^9-THC disrupt the normal interplay between neurotransmitters in this area and may bear relevance in understanding neuronal mechanisms underlying cannabinoid-induced cognitive deficits

Theme: Neural basis of behavior

Topic: Drugs of abuse: opioids and others

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Cannabis derivatives are among the most widely abused substances for recreational purposes. Their actions are mediated by the stimulation of G-protein-coupled CB1 receptors discretely distributed in the brain [12]. In the prefrontal cortex (PFC) a dense autoradiographic labelling of CB1 receptors was detected. This area is implicated in many cognitive functions, including working memory, temporal organization of behavior, and adaptation of behavioral strategies [10,26]. The activity of pyramidal neurons, the major efferents of the PFC, is regulated by complex interactions between dopaminergic and GABAergic neurons arising from the ventral tegmental area (VTA) [3], glutamatergic cortical and hippocampal efferents and an extensive network of GABAergic interneurons. Cannabinoids have been demonstrated to modulate neuronal inputs impinging on PFC neurons. They augment mesocortical dopaminergic neuronal activity [6] and decrease excitability of GABAergic and glutamatergic neurons in the hippocampus in vitro, where they also reduce the release of GABA and glutamate [15,16]. Moreover, in a recent study we showed that cannabinoids excite pyramidal neurons in the PFC and suppress the inhibition evoked by the electrical stimulation of the VTA [24], while Ferraro et al. [7] demonstrated that the synthetic cannabinoid agonist WIN 55,212-2 increases glutamate levels in the PFC. Since cannabinoids have also been demonstrated to induce deficit in learning and memory processes in humans [25] and rats [20–22], we hypothesized that these behavioral effects may be produced, at least in part, by an imbalance in neuronal transmission in the PFC. To test this hypothesis, in this study we sought to determine whether cannabinoids are able to influenc extracellular levels of

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dopamine (DA), GABA and glutamate in the PFC, under identical experimental conditions, by utilizing in vivo brain microdialysis.

Male Sprague–Dawley albino rats (Harlan, Italy) weighing 250–280 g were used. All animals were kept on a 12/12-h light/dark cycle (lights on at 07:00 h) with food and water available ad libitum. Experimental protocols were approved by the Ethical Committee of the University of Cagliari and performed in strict accordance with the E.C. regulations for the care and use of experimental animals (86/609/EEC).

Rats were anesthetized with Equithesin (5 ml/kg, i.p.) and placed in a Kopf stereotaxic frame. The skull was exposed and a hole was drilled through the two temporal bones for transversal insertion across the PFC (AP +3.0 mm, V −2.7 mm from bregma, according to the Paxinos and Watson atlas [23]) of the dialysis probe (AN 69-HF, Hospal-Dasco, Bologna, Italy; cut-off 20 000 Da, in vitro recovery about 30%).

Dopamine was analyzed after 20–24 h from surgery essentially as already described [9]. Briefly rats were re-anesthetized with chloral hydrate (400 mg/kg, i.p.) and the femoral vein was cannulated for i.v. administration of drugs. Chloral hydrate was supplemented as needed by means of i.v. infusion throughout the experiment. Artificia cerebrospinal flui (composition in mM: NaCl 147, KCl 4, $CaCl_2$ 1.5, pH 6–6.5) was pumped through the dialysis tube at constant rate (2 μl/min). Samples were collected every 20 min and directly injected into a HPLC apparatus. Drugs were administered after collection of at least three consecutive samples differing by no more than 10%.

Glutamate and GABA were analyzed in a different group of rats at least 48 h after microdialysis probe insertion, essentially as already described [7,8]. Rats were anesthetized and processed as above. Sample collection was started at least 3 h after the beginning of perfusion, every 20 min. Each 40-μl sample was spliced in two 20-μl aliquots, and immediately stored at −80 °C until analyzed, separately for GABA and glutamate content.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) in ethanol solution was purchased from Research Biochemicals International (Natick, MA, USA), SR141716A was a generous gift of Sanof Researche (Montpellier, France). Δ^9-THC was evaporated immediately before use under argon gas. Δ^9-THC residue and SR141716A were emulsifie in 1% Tween 80, then diluted in saline solution.

Drugs were administered in a volume of 1 ml/kg. Ten minutes before Δ^9-THC or vehicle (1 ml/kg) administration, SR141716A (1 mg/kg) or vehicle was injected intravenously, and three groups of rats were studied: vehicle-Δ^9-THC, SR141716A-Δ^9-THC, SR141716A-vehicle.

All data were presented as a percentage of the average of three basal samples (100%). Statistical significanc was evaluated by one-way analysis of variance (ANOVA) for repeated measures, followed by Dunnett's test as post-hoc.

In the rat PFC, basal GABA, glutamate and DA levels were not significantl different between the treatment groups (GABA: $F(2,18)=0.9268$, glutamate: $F(2,16)=0.7712$, dopamine: $F(2,13)=0.8832$), as indicated in Table 1. Due to technical problems, dialysates from two rats (one belonging to the SR-Δ^9-THC group and one to the SR-vehicle group) were not analyzed for glutamate content.

As shown in Fig. 1A, following Δ^9-THC administration (1 mg/kg, i.v.), extracellular GABA levels rapidly decreased down to 80±4% ($F(8,48)=2.862$, $P<0.05$; $n=7$) of basal levels, returning to basal values after 1 h. This Δ^9-THC induced decrease was completely antagonized by the previous administration of SR 141716A (1 mg/kg, i.v.; $F(8,48)=1.117$, $P>0.05$; $n=7$). At this dose, SR 141716A per se was devoid of any effect on extracellular GABA levels ($F(8,48)=0.275$, $P>0.05$; $n=7$) (Fig. 1A).

On the other hand, PFC extracellular glutamate levels were significantl increased after Δ^9-THC administration (1 mg/kg, i.v.) up to 124±4% ($F(8,48)=9.247$, $P<0.001$; $n=7$) (Fig. 1B). This increase was rapid, short-lasting and completely antagonized by previous administration of SR 141716A (1 mg/kg i.v.; $F(8,40)=0.875$, $P>0.05$; $n=6$). When administered alone, this SR 141716A dose did not induce any significan effect on glutamate levels ($F(8,40)=1.963$, $P>0.05$; $n=6$).

In a different group of rats, dopamine extracellular levels were increased by Δ^9-THC administration up to a maximum of 167±11% ($F(8,40)=23.999$, $P<0.001$; $n=6$) of basal values. As already shown for GABA and glutamate, SR 141716A administration was per se ineffective on DA level ($F(8,40)=2.13$, $P>0.05$; $n=6$) but antagonized the Δ^9-THC induced increase in DA levels ($F(8,24)=2.108$, $P>0.05$; $n=4$) (Fig. 1C).

The administration of a vehicle (1 ml/kg, i.v.) to control animals, did not modify extracellular GABA, glutamate and DA levels in the PFC (data not shown).

Table 1
GABA, glutamate and dopamine basal levels in the rat prefrontal cortex

Group of experiments	GABA	Glutamate	Dopamine
Vehicle+Δ^9-THC	29.76±9.02 (7)	3833.5±1062.0 (7)	79.95±7.85 (6)
SR+vehicle	27.26±3.75 (7)	2494.1±684.0 (6)	70.38±7.69 (6)
SR+Δ^9-THC	39.02±5.38 (7)	3736.2±879.8 (6)	64.58±8.53 (4)

Values are expressed as nmol/l, and are the mean±S.E.M. of the number of rats indicated in parenthesis. No significan difference was found between groups.

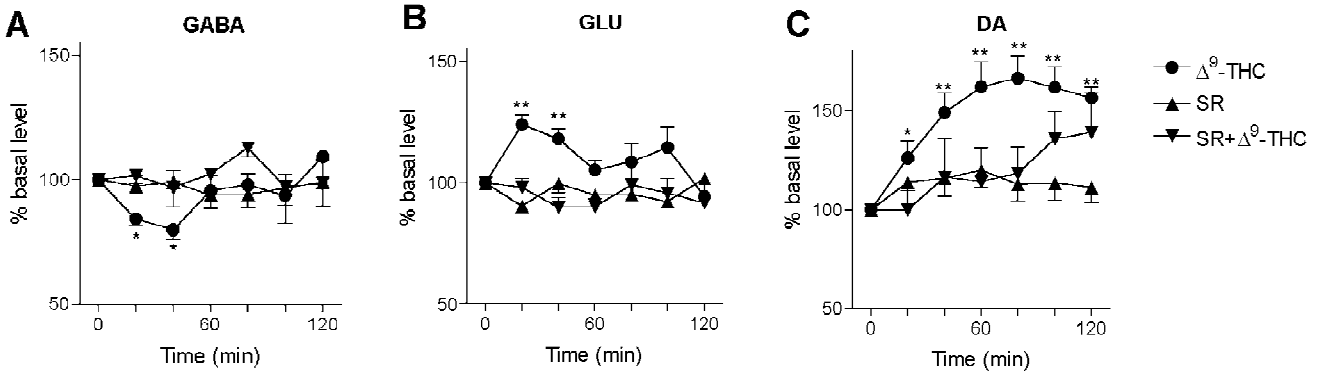

Fig. 1. Δ^9-THC (1 mg/kg i.v.) decreases extracellular GABA (A), and increases glutamate (GLU) (B) and dopamine (DA) (C) levels in the prefrontal cortex. These effects were prevented by the previous administration of SR141716A (1 mg/kg, i.v.) (SR+THC). SR141716A (SR, 1 mg/kg i.v.) per se did not affect either neurotransmitter level. Values are the mean±S.E.M. and are expressed as percent of mean basal level. *$P<0.05$, **$P<0.01$ with respect to basal values (one-way ANOVA, Dunnett's multiple comparison test).

This study was designed to investigate whether Δ^9-THC influence extracellular levels of DA, GABA and glutamate in the PFC under identical experimental conditions. The present data indicate that Δ^9-THC decreases extracellular GABA and increases DA and glutamate levels in the PFC of anesthetized rats. These effects were CB1 dependent, since the CB1 receptor antagonist/reverse agonist SR141716A prevented them. This compound per se did not affect neurotransmitter levels in this brain area at the dose tested.

The findin that Δ^9-THC increases DA output in the PFC confirm previous studies that demonstrated an increase of DA release [4] and DA turnover [14] in the PFC following cannabinoid pretreatment. Diana et al. [6] suggested that the augmented DA output in the PFC after Δ^9-THC treatment might be the consequence of the stimulation of the electrical activity of meso-cortical DA neurons induced by cannabinoids.

Previous studies suggested that cannabinoids decrease the excitability of GABAergic interneurons and the release of GABA in the rat hippocampus [15]and cerebral cortex [8]. This possibility is supported by the findin that CB1 receptors are preferentially localized on GABAergic interneurons in these areas. Consistently, we observed a decrease in GABA outflo in the PFC following cannabinoid treatment. We have recently demonstrated that cannabinoids are able to increase the excitability of PFC neurons projecting to the VTA, and to suppress the inhibitory effect of VTA stimulation [24]. In this paper we provide evidence for one of the possible mechanisms underlying those observations, since the excitation of PFC pyramidal neurons may be due to cannabinoid-induced decrease of GABA release from intrinsic and/or extrinsic GABAergic neurons.

Interestingly, in this study, an increase in glutamate outflo parallels the decrease in extracellular GABA. These finding are consistent with those by Ferraro et al. [7], whereas are in contrast with in vitro studies demonstrating a decrease rather than an increase in glutamatergic transmission in the cerebellum [18], hippocampus [27] and in the PFC [1] after cannabinoid treatment. In our study we measured glutamate levels in vivo following Δ^9-THC administration, therefore it is plausible that these discrepancies may be due to different experimental conditions, since afferent excitatory connections arising from other brain areas or from adjacent parts of the neocortex are lost in slices. Several hypotheses may explain the increase in glutamate in vivo: one possibility is that part of the released glutamate may derive from recurrent axon collaterals of pyramidal neurons that are excited by cannabinoids [24]. The other possibility is that different glutamatergic inputs arising from adjacent cortical areas [2], hippocampus [11] or subcortical structures [17] may be potentiated by Δ^9-THC.

Understanding the reciprocal interactions between the principal neurotransmitters in the PFC may also help us to unravel the mechanisms underlying specifi pathological conditions, such as schizophrenia, in which disruptions of PFC functioning plays a major role. In this respect, the action of cannabinoids may bear a particular relevance, since it is debated whether their heavy abuse may precipitate psychotic episodes in some individuals [13]. Indeed the link between endocannabinoid system and schizophrenia has been suggested by recent studies [5,19].

Taken together, our results suggest that the PFC neuronal activity may be disrupted by cannabinoids by altering neurotransmitter levels in this area, thus indicating a possible neuronal substrate underlying some of the behavioral effects induced by these drugs.

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